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Original article

Artificial modification of the chemical composition of orange oil (*Citrus sinensis* L.) and its effect on larvicidal activity

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ABSTRACT

The use of synthetic pesticide carries along several disadvantages talking about the preservation of the natural homeostasis of the planet, causing the searching of biopesticide, which one presents advantages as well as biodegradability in minimum possible time, the low toxicity in comparison to synthetic pesticides and their variety of structure, which allows slowing down the appearance of resistance. The aim of this work was to evaluate the effect on the larvicidal action when artificially varying the chemical composition of orange oil (*Citrus sinensis* L.). As results, we found that the analysis of gas chromatography coupled to mass spectrometry showed the presence of terpenoid and sesquiterpenoid compounds in the different samples. The use of electric pulses on samples modified their chemical composition, so that the percentage of limonene went from 72% in the sample that was not subjected to electric treatment to lower percentages, even in sample three the percentage of limonene was <50%. Only three compounds (limonene, linalool and caryophyllene) were found to be common in all samples. Subsequently, the larvicidal action on *Drosophila melanogaster* larvae was evaluated. Six concentrations of each oil sample were tested (0, 100, 500, 1000, 5000 and 10000 ppm). We found that there was no linear relationship between concentration and lethality. Additionally, in the sample without electrical treatment most of the concentrations tested had lethality higher than 50%, while in sample 7 the results of the lethality were lower than 30%, so that biological tests showed that in samples where the concentration of limonene was lower, the lethality in the larvae decreased.

1. Introduction

In our modern processes of agriculture, an extensive use of pesticides has been made; this use has brought a series of consequences that go from environmental to health problems. The presence of pesticide residues in agricultural use ground is increasing every time. Due to these kind of problems pesticides constitute an environmental risk and an important source of human exposition (Rojas et al., 2019; Mostafalou and Abdollahi, M., 2017). This fact has resulted in an increase in the type of analytical techniques used in the detection of agricultural chemical residues to make a better controller of crop products (Garcia et al., 2017; Morena, 2015), and their impact on humans and the environment. In addition, the use of pesticides has the consequence that many organisms, such as insects, become resistant to their use. For this reason, today more environmentally friendly alternatives are being developed around the world to try to mitigate the adverse effects of pesticides in the environment. Many of these alternatives have consisted of using natural products, such as plant extracts and essential oils instead of these agrochemicals (Pavela and Benelli, 2016; Abdelli et al., 2016).

Essential oils are secondary metabolites bio-synthesized by plants (Pandey et al., 2017), produced as defense mechanisms to

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environmental responses and ecological factors. Due to essential oils with low mammalian toxicity, fully biodegradable, multifunctional and environmentally safe, there is increasing interest in research concerning with the naturally occurring toxicants from plants as new alternative pesticides (Zhang et al., 2016). The larvicidal activity of numerous essential oils has been the subject of several investigations in search of novel strategies for insect control (Peixoto et al., 2015; Kumar, et al., 2012). It has been proved that essential oils from 1500 plant species have insecticidal properties and are efficacious regarding both forms of insects—adults and larvae (Jankowska et al., 2018). One of the plant species studied is *Citrus sinensis* L. which is a shrub, or more precisely, a tree; from which different aromatic products can be obtained, depending on the part used It belongs to the Family: Rutacea, Genus: *Citrus*. (Etebu and Nwauzoma, 2014). Orange peel has been reported as an excellent substrate for various value-added products, such as essential oils, pectin, natural antioxidants, antimicrobial, ethanol, organic acids and pectotic oligosaccharides (Mamma and Christakopoulus, 2014). Orange essential oil is found mainly in oval-shaped sacks on the flavedo or orange portion of the peel and acts as a natural toxic barrier for many microorganisms and insects (Ceron-salazar and Cardona-Alzate, 2001). The essential oil obtained from orange peel (*Citrus sinensis* L.) has been subjects of several researches due to its functionality as antimicrobial as has been demonstrated in several recent studies such as the work of Geraci et al., in 2016. In this research for example was found as main component, d-limonene (73.9–97%) in orange peel (Geraci et al., 2016); Xiao et al., in 2016, mention that compound such as alpha pinene, sabineno, limonene, δ-terpinolene, hexanal, octanal, decanal, y dodecanal, were typical aromatic compound, that had a covariance with a characteristic smell of sweet orange essential oil. These data are founded through the characterization of active compounds by gas chromatography coupled to mass spectrometry (GC–MS) (Xiao et al., 2016; Minteguiaga et al., 2017). According to the previous investigations and knowing that the terpenes are classified as ingredients bio-active with a variant mode action larvicide, regulators of growth insects, repellents and toxics (Khare et al., 2019). Considering the presence of this type of compound in the essential oils of the orange peel and the consequent use of these as a bioactive insecticide (Carmona et al., 2014). The main objective of the research was to artificially modify the chemical composition of orange oil (*Citrus sinensis* L.) using electrical pulses (Gitonga et al., 2018; Liu et al., 2014). By analysis gas chromatography coupled to mass spectrometry, it was found that the electrical treatment modified the composition of the different samples of treated oils. Additionally, the larvicidal activity of the oils on *Drosophila melanogaster* larvae was evaluated in order to establish the relationship between chemical composition and lethality.

2. Materials and methods

2.1. Supply and characterization of essential oils

The samples of essential oils of *Citrus sinensis* L. extracted from de orange peel were supplied by the company Logibell S.A.S located in Sincelejo city (Sucre -Colombia). This company provided a total of eight samples of essential oils; one sample that was extracted by conventional methods that was used as a reference, and seven samples of orange oils that were subjected to artificial modification of their chemical composition using electrical pulses and the company's home made methods. Orange essential oils were treated on a pilot scale, using a continuous pulsed electric field (PEF). The orange oils were pumped through the treatment chamber to subject the PEF treatment to 30 $kVcm^{-1}$–50 $kVcm^{-1}$. 100 keystrokes were applied; using continuous pulses for 2 h. To check that there was a difference in the chemical composition of the different oil samples supplied, the refractive index was calculated and a GC–MS analysis was performed as described in the following sections.

2.1.1. Essential oils characterization

The refractive index was measured on a Sperscientific 300034 refractometer, measurement at constant temperature was guaranteed, each value is the average of five measurements, with a temperature of 22 °C.

2.1.2. Chemical analysis by gas chromatography coupled to mass spectrometry (GC–MS)

The analysis of all essential oils was performed using a gas chromatography–mass spectrometry (GC–MS) system consisting of an Agilent Technologies 7890A gas chromatograph coupled to a 5975 mass spectrometer. The chromatographic separation was carried out using an HP-5 capillary column (30 m × 0.25 mm i.d. × 0. 25 μm film thickness). The initial column temperature of 45 °C was held for 2 min and increased by 3 °C/min to 280 °C; the temperature was then held there for 30 min. Helium was used as the carrier gas at a flow rate of 25 mL/min. The injector temperature was maintained at 250 °C. The samples were injected at 1 μL in split mode. The percentage composition was calculated by integrating the peak areas of the spectrograms.

2.2. Biological essays

Canton S Strains of *Drosophila melanogaster* were kept in a glass jar of 250 mL of capability with growing medium prepared on base of the mixture composed by 53 g of sugar, 64 g of corn flour, 8.4 g of agar-agar, 22 g of active yeast, in a totality of 1000 mL of water; the mixture was subjected to cooking with controlled times, as a preservative will be used 5 mL of acetic acid to the 45% p/v and 5 mL de nipagin. To allow the exchange of gases, the closure system was made of cotton wrapped in gauze. These glass jars were kept at environmental temperature of 24 °C and with a relative humidity of 57% measured with a thermohygrometer of reference HTC – a photoperiod of 10 h/14 h L/O.

For the genetic homogenization of the strain, through the crossing of five "parental" couples (P), to obtain a first generation (F1) once the progeny are obtained parents eliminated leaving only F1 for crossovers. To obtain (F2) at least ten pairs of (F1) were taken and crossed, where again the parents were eliminated before the adults of the new generation emerged in order to avoid crossing individuals of two different generations. This is how it was obtained (F3), this progeny was used for the experiment, and thus it was guaranteed that the mortality was only due to the toxicity of the treatment that was applied (Suazo et al., 2012).

2.2.1. Larvicidal activity essays

2.2.1.1. Lethality essay with positive control. The bioassay of essential oils, groups of 50 larvae (2nd stage) of *Drosophila melanogaster* were used (Panchal and Tiwari, 2017); in order to determine LC_{50} were exposed for 7 days to various concentrations of imidacloprid (0, 10, 100, 1000 and 10000 ppm) mixed to the diet. 50 μL solution of the compound was mixed with 4 g of the diet and placed in Petri dishes. The number of dead and alive flies was recorded daily for 7 days. A control diet was treated with 50 μL of ethanol. Treated and control insects were held at the same condition used for colony maintenance. For each concentration, we performed tests three times. These treatments contemplated two repetitions in the shortest time to reduce the experimental error (Park et al., 2004).

2.2.1.2. Lethality essay using essential oils. The procedure described above for selection and treatment of larvae was used. Concentrations of 0, 100, 500, 1000, 5000 and 10000 ppm were tested for each oil sample; 50 μL solution of each oil sample was mixed with 4 g of the diet and placed in Petri dishes. A control diet was treated with 50 μL of ethanol. The number of dead and alive flies was recorded daily for 7 days (Suazo et al., 2012). Treated and control insects were held at the same condition used for colony maintenance. For each concentration, we performed tests three times. These treatments contemplated two repetitions in the shortest time to reduce the experimental error (Park et al., 2004).

The results are presented as percentage of mortality (Eq. (1)) (Vargas-Mendez, et al., 2019)

$$\%\text{Mortality} = \frac{\text{totaldiedlarvae}}{\text{totallarvae}} \times 100 \quad (1)$$

2.3. Statistics analysis

Lethal concentrations (LC) at 25 and 50% of each sample of positive control were calculated using SPSS software and probit analysis (Finney, 2009). The data are reported as the sample mean ± standard error. The normal distribution was checked with Shapiro-Wilk followed by Kruskal-Wallis tests and Dunn post test to determine differences between samples, with statistical differences established at $P < 0.05$, using GraphPad Prism (Vecchio et al., 2012).

3. Results

3.1. Essential oils characterization

The chemical characterization of the different samples of essential oils was carried out in order to check that the oils supplied by Logibell Company presented different chemical composition. All oil samples were liquid at room temperature, were volatile, colorless and have density less than unity. Table 1 shows the results for the refractive index of the analyzed samples, finding that there are differences from the third significant number in the index of refraction, which indicates that there may be changes in the chemical composition of the oils, due to the electrical treatment to which the oils were subjected. A more precise analytical technique was used to corroborate this phenomenon. The following section shows the results of gas chromatography coupled to mass spectrometry analysis.

Table 1
Refractive index of samples of oils of the species *Citrus sinensis* L.

Number of the sample	Sample name	Refraction index
1	Orange oil process variant 1 (Refining 1)	1.4711
2	Orange oil process variant 2 Refining 2)	1.4709
3	Orange oil process variant 4 (Refining 4)	1.4725
4	Orange oil process variant 5 (Refining 5)	1,4713
5	Orange oil process variant 6 (Refining 6)	1.4710
6	Orange oil process variant 10 (Refining 10)	1.4717
7	Orange oil process variant 23 (Refining 23)	1.4691
8	Orange without process variant	1.4718

Chromatographic analysis of essential oil samples extracted from *Citrus sinensis* L. revealed the presence of mainly terpenoid-type compounds, as is shown in Table 2.

According to Table 2, it was found that most of the compounds present are monoterpenes and sesquiterpenes, which is in agreement with what is reported in the literature for this type of samples; being found that the compounds limonene, linalool and caryophillene appear in all the samples, while the limonene oxide, *cis*-carveol, α-copaene, β-elemene, β-cubenene, citronellol, terpineol and β-citral appear in most of the samples. According to Table 2, sample 8, which was not subjected to electrical treatment, presents the highest percentage of limonene (72.0%) and the only sample where the presence of carvacrol (0.5%), valencene (0.5%) and nuciferol (0.9%) was detected; physically this sample showed the most yellow coloration and greater odor was perceived with respect to the treated samples. Although in sample 1 the concentration of limonene (64.9%) and linalool (3.9%) decreases with respect to sample 8; the concentration of *cis*-carveol (4.9%) and limonene oxide (2.2%) are the highest in the samples studied; while *p*- Mentha-2,8-dien-1-ol (0.9%) and 1-decanol (3.5%) were only detected in this sample. Sample 2 has the closest percentage of detected metabolites to those reported in the reference sample and is the only one in which the presence of 2,4-Undecadien-1-ol (2.4%) was detected. The most significant decrease in the percentages of limonene (49.1%) and linalool (2.9%) with respect to the reference sample was found in sample 3, but at the same time it presents the highest concentrations of α-copaene (2.9%); β-elemene (7.1%), caryophyllene (5.5%), eremophyllene (2.2%), spathulenol (3.4%), Caryophyllene oxide (1.2%) and cadinene (1.2%); the presence of decanal and α-humulene was detected exclusively in sample 3, and this sample presented the lowest odor and was more translucent. Sample 4 was characterized by presenting the highest percentages of citronellol (1.6%), 1,6-dihydrocarveol (3.0%) and β-pinene (3.1%). Terpinen-4-ol and thymol only appeared in this sample. In sample 5 there was a high percentage of rhodinol (4.0%) compared to the other samples. The presence of 8-hydroxilinalool (1.0%), myrtenol (1.5%), isopinocamfone (1.6%) and gurjunene (0.4%) was detected exclusively. A high percentage of terpineol (14.6%) was detected in sample 6 and was the only sample where 3-carene (0.4%) and decanoic acid (2.8%) were detected. Finally, the highest percentage of linalool (6.4%), β-citral (1.8%), 2-undecenal l (2.8%) was found in sample 7. The presence or absence of some compounds is due to the fact that they are generally intermediaries in the biosynthetic routes of other metabolites present in orange oils.

3.2. Larvicidal activity

3.2.1. Essay with positive control

Imidacloprid, belonging to the neonicotinoid family (Denecke et al., 2017) was used as a positive control. The LC_{50} value found for the *Canton S* strain was 16.395 ppm. This result indicates that the strain is sensitive to the xenobiotic used and allows us to have a reference point to compare the lethality of different oil samples.

3.2.2. Larvicidal assay

The lethality results of the different samples are presented in Table 3; was found that the lethality results in *Drosophila melanogaster* larvae are not better than the lethality presented by the positive control, that is, the artificial variation in the chemical composition following the protocol of the company Logibell S.A.S does not improve the larvicidal action of orange oils. This is demonstrated by the fact that the sample that was not subjected to artificial variation in its composition (sample 8) presented higher lethality values than the other samples, except for sample number 3 at concentrations of 500 ppm and 5000 ppm. Statistically

Table 2
Chemical composition of the orange oils supplied by Logibell Company.

		Content (%)							
N°	**Compounds**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8** (Without process variant)
1	Limonene	64.9	71.2	49.1	70.7	63.8	65.3	65.4	72.0
2	Linalool	3.9	3.8	2.9	3.3	4.9	3.0	6.4	4.2
3	*p*- Mentha-2,8-dien-1-ol	0,9	–	2.9		–	–	–	–
4	Limonene oxide	2.2	0.9	–	0.7	0.7	–	0.4	0.5
6	1-Decanol	3.5	–	–	–	–	–	–	–
6	*cis*-Carveol	4.9	0.8	3.3	1.0	–	0.4	–	0.6
7	Perillaldehyde	0.5	–	–	–	–	–	–	–
8	α-Copaene		0.3	2.9	0.5	0.2	0.2	–	0.2
9	β-Elemene	0.5	0.4	7.1	1.8	0.3	0.2	–	0.2
10	Caryophyllene	0.6	0.5	5.5	1.3	0.6	0.2	0.3	0.5
11	β-Cubebene	0.3	0.2	0.5	–	0.2	0.3	–	0.1
12	Eremophyllene	1.5	1.3	2.2	1.2	–	–	0.7	–
13	Cadinene	0.4	0.3	1.2	0.4	0.2	–	–	–
14	n-Heptacosane	0.2	0.1	–	0.1	0.1	0.1	–	–
15	Heneicosane	0.2	0.2	0.1	0.1	0.1	0.1	–	–
16	Citronellol	–	0.8	0.9	1.6	1.1	–	0.8	0.4
17	Terpineol	–	2.9	0.8	0.4	–	14.6	8.3	3.9
18	β-Citral	–	1.4	–	0.5	0.6	–	1.8	0.9
19	2,4-Undecadien-1-ol	–	2.1	–	–	–	–	–	–
20	Dodecanal	–	0.4	–	–	–	–	–	0.2
21	β-Famesene	–	0.2	–	–	–	0.2	–	–
22	1,6-Dihydrocarveol	–	–	0.5	3.0	–	0.6	–	–
23	Decanal	–	–	3.7	–	–	–	–	–
24	Rhodinol	–	–	0.9	0.9	4.0	–	–	0.8
25	Carvone	–	–	1.0	–	0.4	0.4	–	–
26	2-Undecenal	–	–	1.7	–	–	–	2.8	–
27	α-Humulene	–	–	1.3	–	–	–	–	–
28	Gamma-Muurolene	–	–	0.5	0.3	–	–	–	–
29	Caryophyllene oxide	–	–	1.2	0.2	–	–	–	0.1
30	Spathulenol	–	–	3.4	0.4	0.2	–	–	–
31	Phytol	–	–	0.1	0.1	–	–	–	–
32	α-Pinene	–	–	–	0.1	0.1	0.2	–	0.2
33	β-Pinene	–	–	–	3.1	2.2	2.2	–	3.0
34	Cymene	–	–	–	0.3	–	–	–	–
35	Terpinen-4-ol	–	–	–	0.9	–	–	–	–
36	*cis*-Geraniol	–	–	–	0.3	–	–	–	1.8
37	Thymol	–	–	–	0.2	–	–	–	–
38	8-Hydroxilinalool	–	–	–	–	1.0	–	–	–
39	Myrtenol	–	–	–	–	1.5	–	–	–
40	Isopinocamfone	–	–	–	–	1.6	–	–	–
41	Gurjunene	–	–	–	–	0.4	–	–	–
42	9-Dodecen-1-ol	–	–	–	–	0.3	–	–	–
43	Cedrene	–	–	–	–	0.3	–	–	0.4
44	α-Amorphene	–	–	–	–	1.4	0.7	–	–
45	3-Carene	–	–	–	–	–	0.4	–	–
46	Decanoic acid	–	–	–	–	–	2.8	–	–
47	*cis*-Mirtanol	–	–	–	–	–	–	0.9	–
48	Perillyl alcohol	–	–	–	–	–	–	0.4	–
49	Carvacrol	–	–	–	–	–	–	–	0.5
50	Valencene	–	–	–	–	–	–	–	0.5
51	Nuciferol	–	–	–	–	–	–	–	0.9

Table 3
Lethality of oil samples on *Drosophila melanogaster*.

	Concentrations (ppm)					Negative control
Number of sample	**100**	**500**	**1000**	**5000**	**10,000**	**0**
1	28.33 ± 1.67	45.00 ± 5.00	21.67 ± 3.07*	15.00 ± 2.24	43.33 ± 4.22	0.00
2	21.67 ± 1.67*	20.00 ± 2.58*	30.00 ± 4.47	13.33 ± 2.11	10.00 ± 2.58*	0.00
3	21.67 ± 3.07*	60.00 ± 3.65	46.67 ± 4.22	45.00 ± 4.28	53.33 ± 3.33	0.00
4	15.00 ± 2.24*	28.33 ± 3.07	23.33 ± 2.11	10.00 ± 2.58*	36.67 ± 2.11	0.00
5	21.67 ± 4.01*	30.00 ± 4.47	25.00 ± 2.24	30.00 ± 2.58	36.67 ± 4.22	0.00
6	18.33 ± 3.07*	25.00 ± 2.24*	11.67 ± 1.67*	15.00 ± 2.24	26.67 ± 2.11*	0.00
7	13.33 ± 2.11*	25.00 ± 2.24*	6.67 ± 2.11*	15.00 ± 2.24	23.33 ± 2.11*	0.00
8	53.33 ± 2.11	51.67 ± 3.07	56.67 ± 4.22	30.00 ± 4.47	58.33 ± 4.01	0.00

Results are expressed as mean ± standard error.
* Statistically significant difference between samples respect to sample 8 ($p < 0.05$).

it is found that in most treated samples the lethality results are below the average of the untreated sample with electric pulses; sample 8 shows higher lethality at 10000 ppm. From Table 3 it was observed that of the modified samples, sample 1 at 100 ppm

has the highest lethality (28.33%). We found that at 100 ppm the lethality of samples two, three and five are very similar (around 21.67%), the same behavior is observed in samples four and five at 10,000 ppm (around 36.67%); but equal lethality was found in samples six and seven at 500 ppm and in samples one, six and seven at 5,000 ppm. If only the modified samples are compared, sample 3 had the highest lethality at 500, 1000, 5000 and 10000 ppm; while sample 7 had the lowest lethality at 100 and 1000 ppm; sample 2 had the lowest lethality at 500 and 10000 ppm and at 5000 ppm sample 4 had the lowest lethality. We found that the lethality of samples two, four, five, six and seven did not exceed 40% lethality at any of the concentrations tested.

As a final remark, it is observed that in the treatments applied on the *Drosophila* larvae, no linear variation was found between the concentration of the xenobiotic and the lethality.

4. Discussion

In an attempt to find alternatives to replace the use of synthetic insecticides; the company Logibell S.A.S using electrical pulses modified the chemical composition of oils extracted from orange (*Citrus sinensis* L.), in order to test whether these modifications had an effect on the lethality in *Drosophila melanogaster* larvae. Through this innovative approach it was possible to change the proportions in which the metabolites were detected in the treated samples. It was found that the refractive index of the samples was different from the reference sample, indicating that the mole fraction of the compounds present has been altered (Delgado et al., 2016). Although essential oils have been catalogued as potential biological actives; structural modifications of these oils are becoming more common, so in recent years there has been mention of the use of electrical pulses as a means of modifying these substances (Liu et al., 2014; Gitonga et al., 2018).

In order to verify that the modification in the oil samples, a CG-MS analysis was carried out and it was found that the application of electric pulses carried out an effective modification in the chemical composition of the essential oil, since the concentration of the metabolites increased or decreased according to the variation applied, even some new compounds appear in the different samples that had not been detected, possibly due to their low concentration or some of the metabolites act as biological precursors of others. When reviewing the results of the CG-MS, we found the presence of metabolites that act as precursors of others, for example limonene can react in different ways and produce compounds such as carvone, *cis*-carveol, α-terpineol, limonene oxide among others (Villarreal, et al., 2018). Also in the Table 2 there are compounds that through some biosynthesis routes can be precursors of limonene such as pinene, which can also be used for the production of linalool; a similar situation occurs with humulene, which is a precursor of caryophyllene (Carrillo, et al., 2019). The outcomes found indicate that it is possible to develop routes of artificial biosynthesis of metabolites in essential oils, so it is important to further optimize this process in order to obtain samples of oils enriched with metabolites that enhance a particular biological activity. Therefore, a possible application of the technology used in this work may be the transformation of monoterpenes into compounds that provide greater chemical stability to those of essential oils, or to achieve an increase in the presence of oxygenated metabolites that can give rise to more water-soluble oil. Advances in technology of this type may lead to future alternatives in insect control and even commercial applications such as the manufacture of more stable oils with better organoleptic characteristics and greater commercial value.

Taking into account that the objective of the work was to check if the modifications in the chemical composition of the orange oils caused a variation in the larvicidal activity; the bioassay was initiated using imidacloprid as a positive control. The LC_{50} value found for the *Canton S* strain was 16.395 ppm, this result indicates that the strain is sensitive to this insecticide. As shown in Table 3, none of the reported concentrations are close to the lethal concentration 50 of imidacloprid. Nevertheless, mortality rate for all oils was low prevented estimation of LC_{50} in all cases. Therefore, presently investigated in this work essential oils are likely ineffective as natural insecticide. According to the above, it is recommended to continue testing variations in the refining processes through the use of electric pulses in order to obtain oils with a higher biological activity with lethality comparable to the reference synthetic insecticides.

In this contribution we found that all the studied samples are rich in terpenoid compounds, whose majority metabolite in most of the samples was limonene, from which there are commercially available larvicides (Showler et al., 2019). A LC_{50} value of 2.18 ppm has been reported for limonene in *Drosophila melanogaster* (Zhang et al., 2016), these authors reported the lethal 50 concentrations for the following monoterpenes: linalool (0.35 ppm), β-citronellol (0.82 ppm), terpineol (0.31 ppm) carvone (0.54 ppm), α-pinene (4.12 ppm), and β-pinene (1.41 ppm). Knowing the LC_{50} values and the concentrations of metabolites reported in Table 2, it was observed that by decreasing the limonene concentration, the larvicidal action decreased (see Table 3). As discussed above, the application of electric pulses caused changes in the proportion of the appearance of metabolites in the different samples. These changes caused a modification in the larvicidal action of the oils; since it is possible to favor the concentration of metabolites with lower LC_{50} values or to cause the presence of compounds with chemical activity that protect the larvae. Activity of essential oils depends on complex interaction among their compounds, which might exhibit additive, synergistic or antagonistic effects (Mitic et al., 2018). Sample 3 presents a different behavior from the other modified samples because although it decreases the concentration of limonene to 49.1%, the larvicidal action does not decrease, in fact the highest larvicidal action was found in this sample at 500 ppm. The possible loss of larvicidal activity by reduction in the concentration of limonene could be compensated by an increase in the concentration of the following sesquiterpenes: caryophyllene (5.5%), α-humulene (1.3%), β-elemene (7.1%) and α-copaene (2.9%); metabolites to which biological activities have been reported as insecticide *in Drosophila melanogaster* (Case et al., 2003; Bucio et al., 2016); controlling dengue and vectors of mosquitoes of *filariasis* a stephensi, ae. *Aegypti* and cx *Quinquefasciatus* (Govindarajan et al., 2016); as a potential larvicide against *A aegypti* (Neves et al., 2017). Other studies highlight the role that plays together with the cariofilene and alpha- hemulene, affirming a synergic action between these being the first one a potentializer of the biological activity associated with the second one (Suazo et al., 2012). According to what has been exposed, it is important to recognize that the application of electrical pulses to the different samples can cause the appearance of compounds with physicochemical characteristics that enhance or decrease the biological activity according to the variants applied to the oils modification process.

Finally, it was observed that the relationship between lethality and the concentration applied to the larvae did not follow a linear behavior, this phenomenon has been observed in insects where the concentrations can stimulate the response mechanism according to the effect caused to their homeostasis, product of the xenobiotic used, this tends to be related to an adaptive response (Raymond and López-Martínez, 2020); the non-linearity can also be explained because the orange oil samples may contain metabolites that act as antagonists of other metabolites, and the biological activity may vary according to the concentrations of the metabolites present.

In conclusion, it was found that the artificial modification in the chemical composition of orange oils causes variation in their larvicidal action; noting that the biological activity could depend in direct relation to the concentration of limonene present in the samples, since when the concentration of limonene decreased, a decrease in lethality was observed; For this reason, the oil sample that was not subjected to treatment with electric pulses generally showed greater larvicidal action on *Drosophila melanogaster* when compared to the modified oils used in this study.

Author contributions

J. Anaya-Gil and R. Gaitán-Ibarra conceived and designed the study; A. Cabarcas-Caro, M. Leyva-Ricardo, J. Parra-Garrido and R. Vivas-Reyes performed the biological evaluation; J. Anaya-Gil, R. Gaitán-Ibarra, R. Vivas-Reyes wrote the first draft of the paper; and A. Cabarcas-Caro, M Leyva-Ricardo and J. Parra-Garrido critically reviewed the paper. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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